INVITED REVIEW

Regulation of allergic inflammatory responses by inhibitory receptors

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Summary

An intricate network of activation and inhibitory signals tightly regulates immune responses. To date, multiple activation receptors have been described. These include receptors that mediate cellular functions such as adhesion, chemotaxis, cytokine signalling, mediator release, survival and phagocytosis. In contrast to these activation pathways, an opposing and suppressive receptor system has evolved. These receptors can override the signals elicited by the activation pathways and are broadly termed inhibitory receptors. Inhibitory receptors share unique intracellular signalling motifs and have key roles in various cellular and pathological conditions. Therefore, such receptors are potential targets for future therapeutics. In this review, we will discuss the structure and function of inhibitory receptors. In particular, we will focus on the expression and function of inhibitory receptors on mast cells and eosinophils and illustrate strategies for their inhibition in the settings of allergic inflammation.

Keywords cytokines, eosinophils, immunoreceptor tyrosine-based inhibitory motif (ITIM), inflammation, inhibitory receptor, mast cells, myeloid cells
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Introduction

An immune response is a common term that describes complex interactions between cells and mediators, as well as mechanisms that act to prevent anti-self reactivity or hyperactivation. Indeed, an intricate network of positive and negative signals mediates the regulation of immune responses, which provides the basis for immune cell homeostasis in health and disease [1]. Integration of such signals is frequently achieved at the cellular level through a combination of signals from a variety of pathways including cell surface receptors, intracellular signalling intermediates and gene transcripts [2].

Studies on the immune system have traditionally focused on pathways that activate immune cell function in response to antigens and pathogens. However, it has recently become apparent that in addition to receptors that stimulate immune cell activation, inhibitory receptors serve as a counter-regulatory system, which can maintain cellular homeostasis [3]. Evidently, loss of inhibitory signalling may result in auto-reactivity and unbalanced inflammatory responses, emphasizing the involvement of such receptors in the regulation of immune responses [1–3].

The term 'inhibitory receptor superfamily' was first set by Lanier in his description of receptors that suppress the activation of natural killer (NK) cells [4]. Although inhibitory receptor function has received a detailed analysis in NK cells, our knowledge about these receptors in the myeloid cell lineage and especially their roles in allergic inflammatory settings has received limited attention [5].

The focus of this review will be on the expression and function of inhibitory receptors in myeloid cells with a specific emphasis on mast cells and eosinophils. Basic structure and mechanism of action will be discussed and a summary of recent advances will be illustrated. Finally, the concept of utilizing inhibitory receptors as pharmacological targets for immune suppression will be presented and discussed.

Inhibitory receptors: structure

Inhibitory receptors can be divided into two groups, belonging either to the immunoglobulin receptor superfamily or to the C-type lectin inhibitory receptors [1, 4, 6]. The Ig superfamily is characterized by a single V-type Ig-like domain in the extracellular portion and may contain

various C-type domains. Myeloid cells express various Ig superfamily receptors including leucocyte Ig-like receptors/Ig-like transcript (LIRs/ILTs now termed CD85a-m) [7], Leucocyte-associated Ig-like receptor (LAIR) [8], gp49B1 [9], CD300 family members [10] and various sialic-acid-binding Ig-like lectins (Siglecs) [11]. The term 'C-type lectin' indicates a calcium-dependent carbohydrate-binding protein motif; yet, many C-type lectin inhibitory receptors possess no obvious calcium binding or carbohydrate specificity [6, 12]. Thus, this term is structural rather than functional. The prototypical C-type lectin inhibitory receptor is the NK receptor NKG2/CD94 or Ly49 [13, 14]. However, myeloid cells express various C-type lectin inhibitory receptors such as mast cell function-associated antigen (MAFA), dendritic-cell-associated C-type lectin 2 (DCAL-2) [15] and dendritic cell inhibitory receptor (DCIR) [12]. C-type lectin receptors have been reviewed extensively elsewhere and therefore will not be discussed in this review [16, 17]. For brevity and clarity of this review, a table summarizing expression patterns of selected inhibitory receptors on myeloid cells is shown (Table 1).

Inhibitory receptors: signalling

The prototype inhibitory receptor (either an Ig superfamily receptor or C-type lectin) can be identified by a consensus amino acid sequence (found both in human and mice), termed the immunoreceptor tyrosine-based inhibitory motif (ITIM), which is present in the cytoplasmic domain of these receptors [1–5]. The ITIM sequence is composed of six amino acids (Ile/Val/Leu/Ser)–X–Tyr–X–X–(Leu/Val), where X represents any amino acid. Inhibitory receptors can express either one or several ITIM domains. Upon engagement with their ligands, the ITIM(s) undergo tyrosine phosphorylation (often by a Src-family kinase), which provides a docking site for the recruitment of cytoplasmic phosphatases having an Src homology 2 (SH2) domain such as SH2-containing phosphates-1 (SHP-1), -2 and SH2 domain-bearing inositol 5-phosphatase-1 (SHIP-1) [18, 19] (Fig. 1). These phosphatases are perceived to dephosphorylate tyrosine residues that provide docking sites for signalling kinases, which are recruited by activation receptors (and therefore suppress signalling) [1–5]. While the majority of inhibitory receptors recruit the tyrosine phosphatases SHP-1 or -2, they usually do not recruit the lipid phosphatase SHIP with the exception of FcγRIIB, which primarily recruits SHIP-1 but not SHP-1 or -2 [20].

It is perceived that optimal inhibition will be achieved with maximal ITIM phosphorylation [21, 22]. Nevertheless, it is unknown whether inhibitory receptors that contain several ITIMs can display a more potent inhibition in comparison with inhibitory receptors that contain fewer ITIMs. Pharmacologically, this may be an important subject to examine as myeloid cells express various

Table 1. Expression pattern of inhibitory receptors on myeloid cells[*]

Inhibitory receptor	Alternative name	Myeloid cell expression
Ig-superfamily receptors		
CD300 family		
CD300a	LMIR-1, CLM-1	MC, Mono, Mac, DC, N, E
CD300f	CLM-8	MC, Mono, Mac, DC, N
Inhibitory human siglecs		
CD33	Siglec-3	Mono, MyoP
Siglec-5	CD170	Mono, MyoP
Siglec-6	CD327	Mono, MyoP
Siglec-7	CD328, p75/AIRM	Mono, MyoP
Siglec-8		Mono, MyoP
Siglec-9	CD329	Mono, MyoP
Siglec-10		Mono, MyoP
Siglec-11		Mono, MyoP
Inhibitory mouse siglecs		
CD33		Mono, Mac, N, E
Siglec-E		N, Mono, Mac, DC
Siglec-F		E
ILTs/LIR family		
Human		
ILT-2/LIR-1	CD85j	Mono, Mac, DC
ILT-3/LIR-5	CD85k	Mono, Mac, DC
ILT-4/LIR-2	CD85d	Mono, Mac, DC, N
ILT-5/LIR-3	CD85a	Mono, Mac, DC, N, E, B
Mouse		
Gp49B1	LILRB4	MC, Mono, Mac, DC, N, E
PIR-B	LILRB3	MC, Mono, Mac, DC, N, E
FcγRIIB	CD32B	MC, Mono, Mac, E, N, DC
LAIR-1	CD305	Mono, Mac, DC
Sirp-1α		
PILRα		Mono
CD200		MC, Mono, Mac
C-type lectin receptors		
DCIR	CLEC4A	DC, Mac, N
DCAL-2		DC
MAFA		MC

[*]This table represents only a partial list of receptors that are expressed on myeloid cells.
MC, mast cell; Mono, monocyte; Mac, macrophage; DC, dendritic cell; N, neutrophil; E, eosinophils; B, basophils; MyoP, myeloid progenitor cell; Siglec, sialic acid binding Ig-like lectin.

inhibitory receptors. Thus, it may be best to target the most 'potent' ones in order to restrain cellular activation.

Although phosphatases are thought to act as the functional arm of inhibitory receptors, there is evidence that an additional non-redundant pathway exists. For example, co-ligation of gp49B1 and FcεR1 on murine mast cells leads to a rapid association of SHP-1 with gp49B1 [21]. Nevertheless, SHP-1-deficient mast cells exhibited only a partial loss of gp49B1-mediated inhibition, suggesting the involvement of other molecular pathways in the counter-regulatory effect, which may be SHP-2 dependent [21]. Thus, it is possible that SHP-1 and SHP-2 possess distinct inhibitory roles. Indeed, recent studies revealed that the
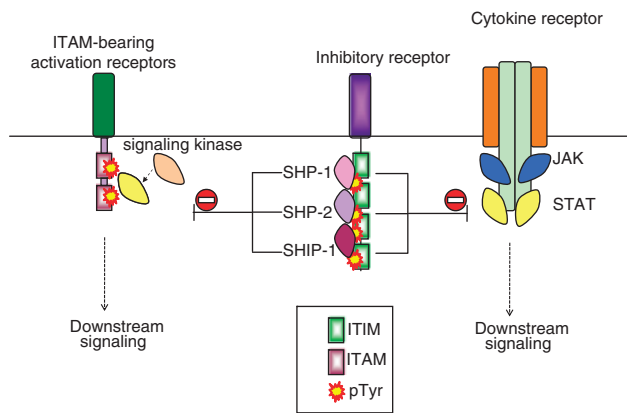
Fig. 1. The mechanism of inhibitory receptor function. Upon binding to their ligand, inhibitory receptors usually recruit an Src-family kinase (e.g. Lyn, Fgr, Hck), which rapidly phosphorylates their intracellular immunoreceptor tyrosine-based inhibitory motif (ITIM) domain. Consequently, Src homology 2 (SH2)-containing phosphatases such as SH2-containing phosphates (SHP)-1, -2 and/or src homology 2 domain-bearing inositol 5-phosphatase-1 (SHIP) bind the receptor and suppress proximal activation signals mediated by other signalling kinases (in *trans*) that are recruited to activation receptors [either via immune receptor tyrosine-based activation motifs (ITAMs) or independent of ITAMs such as cytokine receptors].

differential binding of SHP-1 might have a distinctive inhibitory outcome than the recruitment and binding of SHP-2. This analysis uncovered that SHP-1 is most active when actually bound to the ITIM receptor whereas SHP-2, which possesses a longer spacing between its two C-terminal tyrosines, can be active even when unbound to the ITIM [23, 24]. While the aforementioned studies illustrate distinct structural and functional characteristics of the different phosphatases, inhibitory receptors can mediate their function even in the absence of SHP binding. In fact, upon its phosphorylation, LAIR-1 can bind the c-Src tyrosine kinase (Csk), which is a negative regulator of Src family kinases and can inhibit the B cell receptor-induced activation even in the absence of SHP-1 and -2 [25]. The binding of inhibitory receptors to Csk and the consequent SHP-independent inhibition are likely a shared phenomenon between inhibitory receptors as the ITIM of Ig-like transcript (ILT) 2 and signal-regulatory protein α (SIRP-1α) can also bind Csk [26]. In addition, negative adaptor molecules such as Dok family members may also mediate inhibition that is either mediated via SHIP/SHPs or independently through the direct binding of Dok to Ras GTPase-activating protein (RasGAP) [27–29]. Thus, the downstream molecular mechanisms that are utilized by inhibitory receptors to mediate their function are likely more complex than the traditional concept (i.e. ITIM phosphorylation → phosphatase recruitment → dephosphorylation of kinases → inhibition). The simplistic and traditional view of inhibitory receptor function described the ITIM as an opposing signal to immune receptor tyrosine-based activation motif (ITAM) phos-

phorylation (Fig. 1) [1, 4, 19]. Nevertheless, recent data clearly demonstrate a negative regulation of inhibitory receptors towards receptors that do not contain ITAMs such as adhesion molecules, cytokine receptors, chemokine receptors and innate immune receptors [such as toll-like receptors (TLRs)] [30–37]. An illustration depicting the negative regulation of cytokine receptors is presented in Fig. 1. Collectively, these data indicate a broad role and a complex regulatory mechanism for inhibitory receptors in various immune processes including allergic responses [30, 32, 38].

Allergic response and potential inhibitory checkpoints

Initiation of an allergic response is a process with a distinct hierarchy that involves multiple cell types both of the myeloid and lymphoid origin. The prototypical and conventional allergic response begins with the uptake of an allergen by antigen-presenting cells (primarily dendritic cells) and antigen presentation of allergenic peptides to CD4$^+$ T cells. Subsequently, T cells undergo differentiation into T helper type 2 cells and secrete IL-4 and IL-13, which promote the B cell isotype switching and the generation of allergen-specific IgE. IgE circulates in the blood stream and encounters FcεR1-bearing basophils or tissue mast cells. Upon a second (or repetitive) exposure, allergen binding to IgE results in cross-linking of FcεR1, leading to rapid basophil and mast cell activation. Consequently, an inflammatory cascade begins where leucocytes migrate into the tissue, interact with each other and propagate inflammation and tissue damage via the secretion of various mediators. In the case where inflammation is chronic, irreversible fibrosis may occur leading to a loss of tissue function. This simple illustration demonstrates various molecular checkpoints in which inhibitory receptors can affect the overall immune response in an allergic inflammation (Fig. 2). For example, regulation of dendritic cell function may influence the process of antigen presentation, consequent T cell priming/activation and cytokine profile. Regulation of B cell function and isotype switching may limit IgE levels, and therefore regulate the magnitude of mast cell activation as a secondary outcome. In addition, direct negative regulation of effector cell functions (e.g. mast cells and eosinophils) including degranulation, chemotaxis and survival may determine the extent of their activity, and thus the allergic disease severity. These examples partially illustrate intervention points where regulation of cellular activation by inhibitory receptors can affect the entire allergic cascade and the consequent response (Fig. 2). Indeed, studies examining the function of various inhibitory receptors indicate that they play a role in allergic reactions. For example, studies aimed to define the role of paired immunoglobulin-like receptor B (PIR-B) have demonstrated that PIR-B regulates myeloid cell maturation [39, 40]. Therefore,
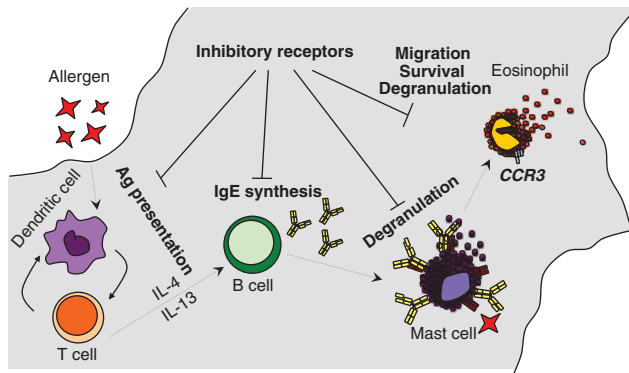
Fig. 2. Cellular checkpoints regulated by inhibitory receptors. The prototype allergic response involves multiple cellular components and various molecular checkpoints that are prone to be regulated by inhibitory receptors. Inhibitory receptors can potentially regulate antigen presentation by dendritic cells, IgE synthesis by B cells, mast cell degranulation and eosinophils migration, survival and degranulation. Loss of negative regulation in any of these molecular checkpoints may lead to excessive immune activation and detrimental consequences.

$Pirb^{-/-}$ dendritic cells remain immature and promote Th2 responses. As such, immunization with T cell-dependent antigens resulted in increased IL-4 and in decreased IFN-γ, as well as enhanced IgG1 and IgE production [40]. Notably, these results could be due to an additive regulation of B cell function by PIR-B as PIR-B has been shown to negatively regulate various B cell responses as well [39, 41, 42]. Likewise, the inhibitory receptor gp49B1, a close homologue of PIR-B, has been shown to negatively regulate inflammatory (primarily eosinophilic) cellular infiltrate and lung pathology in a model of ragweed-induced allergic-airway inflammation [43]. Nevertheless, and despite the aforementioned observations, analysis of inhibitory receptor involvement in experimental allergic disease models has received limited attention.

Although inhibitory receptors can regulate cellular functions of multiple cells involved in allergic reactions, we will next focus on the expression and function of inhibitory receptors in mast cells and eosinophils, as they are hallmark effector cells involved in an allergic response [44].

Inhibition of mast-cell-mediated responses

As mentioned, allergic reactions are characterized by the activation of mast cells and the consequent recruitment of eosinophils. Recent findings suggest that both cell types express various inhibitory receptors [45, 46]. Interestingly, substantially more information exists on the function of inhibitory receptors in mast cells in comparison with eosinophils. This could be due to the availability of several human mast cell lines and the fact that methods for differentiating mast cells from mouse bone marrow are better characterized and widely used than similar methods for eosinophil cultures. In addition, mast-cell-mediated,

IgE-dependent models exist *in vivo* whereas the contribution and relative role of eosinophils in allergic settings is more controversial [47].

Classical studies aimed to define the function of inhibitory receptors on mast cells have largely focused on mast cell degranulation. Studies using platelet-endothelial cell adhesion molecule 1 (Pecam-1)-deficient mast cells demonstrate an increased degranulation in response to IgE-mediated activation [48]. Furthermore, we have shown recently that CD300a on mast cells can suppress IgE-mediated mast cell degranulation. This inhibition was mediated by SHP-1 and SHIP-1 but not SHP-2 and involved the inhibition of Syk and Lyn kinases (Fig. 3a) [49]. Similarly, other inhibitory receptors including FcγRIIB, gp49B1, PIR-B and CD200 have all been shown to suppress mast cell degranulation *in vivo* and *in vitro* [20–22, 29, 50–52]. As such, mast cells deficient in gp49B1 exhibited an increased degranulation and inflammation in models of passive cutaneous anaphylaxis and in active sensitization protocols [53]. In addition to suppressing FcεRI-IgE-mediated responses, mast cell inhibitory receptors can also suppress receptors utilizing the intrinsic kinase activity such as the c-kit, the receptor for stem cell factor (SCF). SCF is a hallmark growth and differentiation marker of mast cells and a key effector of mast cell survival and priming. Thus, a negative regulation of SCF : c-kit-mediated signalling could provide a substantial regulatory pathway in mast cell responses. Interestingly, several inhibitory receptors have been shown to restrain SCF-induced mast cell responses. Co-aggregation of c-kit and FcγRIIB by antibodies in mast cells leads to the inhibition of thymidine incorporation, as well as cell proliferation, which was correlated with cell cycle arrest. This did not affect the ligand-induced c-kit phosphorylation and induced tyrosine phosphorylation of FcγRIIB, which selectively recruited SHIP-1 [54]. Likewise, gp49B1 was found to inhibit SCF-induced tissue swelling resulting from mast cell degranulation. Interestingly, this effect is primarily regulated by the gp49B1-mediated inhibition of cysteinyl leukotriene production by mast cells [55]. Finally, we have shown recently that CD300a is a potent negative regulator of c-kit-induced signalling in mast cells as CD300a activation impaired mast cell differentiation, survival and activation *in vitro*. This effect was mainly derived from the Kit-mediated tyrosine phosphorylation of CD300a and the recruitment of SHIP-1 but not of SHP-1 [56].

Recently, mast cells have been suggested to play important roles in innate immunity [57–59]. Indeed, they express various pattern recognition receptors and are a good source of TNF-α, a potent cytokine implicated in numerous innate immune responses [60–63]. Thus, it is likely that inhibitory receptors expressed by mast cells can regulate their innate immune functions as well. Several lines of evidence (primarily from studies on macrophages
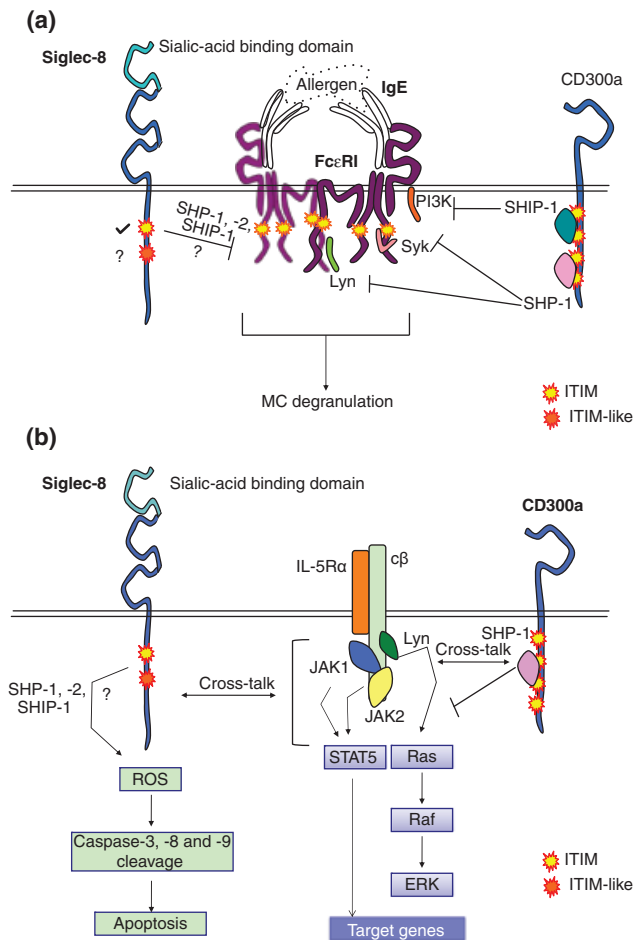
Fig. 3. Comparison of sialic-acid-binding Ig-like lectin (Siglec)-8 and CD300a-mediated inhibition in mast cells and eosinophils. Studies on Siglec-8 and CD300a in (a) mast cells and (b) eosinophils provide a good model to illustrate the similar and different aspects of cellular activation regulated by inhibitory receptors. (a) Both Siglec-8 and CD300a can negatively regulate IgE-dependent mast cell activation. The precise molecular mechanism of Siglec-8-mediated inhibition is not yet clear. However, the proximal immunoreceptor tyrosine-based inhibitory motif (ITIM) domain is essential for this inhibitory response, thus suggesting involvement of SH2-containing phosphates (SHP)-1, -2 or src homology 2 domain-bearing inositol 5-phosphatase-1 (SHIP) in mast cell inhibition. In contrast, the following receptor cross-linking, CD300a interacts with SHP-1 and SHIP-1 but not SHP-2 and suppresses Lyn- and Syk-mediated activation. Nevertheless, the precise involvement and relative contribution of each ITIM is still to be determined. (b) Comparison of Siglec-8 and CD300a effects in eosinophils highlights the differential inhibitory molecular mechanisms. Cross-linking of Siglec-8 induces rapid eosinophil apoptosis that is mediated by the generation of ROS and caspase cleavage. In contrast, cross-linking of CD300a results in the negative regulation of JAK2 and ERK activation that is mediated by the IL-5 receptor complex. Importantly, both Siglec-8- and CD300a-mediated inhibition is amplified by IL-5, hence suggesting an active crosstalk between IL-5 receptor and inhibitory receptors in eosinophils.

and B cells) suggest an important role for inhibitory receptors in the regulation of innate immune responses. For example, PIR-B can suppress macrophage-mediated TLR-2-induced responses and can bind Gram-positive and Gram-negative bacteria [34]. Moreover, PIR-B has been shown to suppress B cell responses to TLR-9 resulting in an augmented autoantibody production [64]. In addition, SIRP-α has been shown to regulate endotoxic shock via the regulation of innate immune responses in macrophages [31]. Zhou et al. [37] have shown elegantly that lipopolysaccharide (LPS)-induced microangiopathy is regulated by gp49B1 primarily by regulating neutrophil- but not mast-cell-mediated innate-immune functions. This is despite the fact that mast cells are fully equipped to respond to LPS and bacteria.

These results highlight the necessity to delineate the relative contribution of inhibitory receptors *in vivo* in disease settings and to define the cellular components that are regulated by such receptors. Furthermore, they call for future functional studies defining the role of inhibitory receptors in the regulation of mast cell-mediated innate-immune responses.

Inhibition of eosinophil responses

As mentioned, functional studies on inhibitory receptor activity in eosinphils are more limited. Although eosinophils have been shown to express several inhibitory receptors, only a few of them have been examined thoroughly. One such receptor is Siglec-8, which is expressed on eosinophils, mast cells and basophils [65, 66]. Co-ligation of Siglec-8 by anti-Siglec 8 antibodies or a polymer expressing its ligand with a secondary antibody has been shown to mediate eosinophil apoptosis by initiating mitochondrial injury, reactive oxygen species (ROS) generation and a rapid cleavage of caspase-3, -8 and -9 [67–71] (Fig. 3b). Of even greater interest is the finding that in the presence of the 'hallmark eosinophil survival cytokines' IL-5 and granulocyte macrophage-colony stimulating factor (GM-CSF), Siglec-8 activity was further enhanced and the requirement for co-ligation of the receptor by a secondary antibody was not needed [68, 69]. Supporting these findings, an administration of anti-Siglec-F antibody (a murine functional paralogue of Siglec-8) in a model of experimental asthma, significantly reduced peribronchial eosinophilic inflammation and subepithelial fibrosis [72]. These results correlated with an increased eosinophil apoptosis in lung and bone marrow [72]. Furthermore, in other studies a single dose of Siglec-F antibody to IL-5 transgenic mice resulted in a rapid reduction in blood eosinophils, which was accompanied by a reduction in tissue eosinophilia [73]. Interestingly and in contrast to its role in eosinophils, Siglec 8 was unable to induce mast cell apoptosis but rather significantly reduced FcεRI-dependent histamine and prostaglandin D2 (PGD$_2$) secretion, as well as FcεRI-dependent contraction of human airway bronchial rings. Importantly, point mutation analysis revealed that this effect

was shown to be dependent on the proximal ITIM of Siglec-8 (Fig. 3a) [52]. In this respect, it is noteworthy that the functional mechanism of Siglecs can differ between various cell types. For example, Siglec-9 has been shown to actively induce neutrophil death via a caspase-dependent (apoptotic) and -independent pathway [74]. Experiments using scavengers of ROS or neutrophils that were unable to generate ROS, indicated that both Siglec-9-mediated caspase-dependent and -independent forms of neutrophil death depend on ROS. Interestingly, the caspase-independent death pathway in neutrophils was characterized by cytoplasmic vacuolization and several other non-apoptotic morphologic features [74].

Similar to studies with Siglec-8, CD300a was also shown to suppress eosinophil survival (Fig. 3b) [75]. However, in contrast to Siglec-8, CD300a does not actively induce apoptosis but rather prevents IL-5- and GM-CSF-mediated survival signals. Indeed, CD300a was shown to suppress JAK2 phosphorylation and therefore may be suppressing the function of the common β-chain of the IL-5, IL-3 and GM-CSF receptor complex [75]. Remarkably (and analogous to Siglec-8), increasing the concentrations of IL-5 and GM-CSF enhanced the inhibitory activity of CD300a [75]. The different outcome of Siglec-8 activation on eosinophils (induction of caspase-dependent apoptosis) as opposed to IRp60/CD300 activation (inhibition of survival signals) may be due to the fact that Siglec-8 contains both ITIM and ITSM (i.e. switch) motifs, which may recruit adaptor molecules such as the signalling lymphocytic activation molecule (SLAM)-associated protein (SAP) containing an SH2 domain (SAP) and/or Ewing's sarcoma-activated transcript-2 (EAT-2) [76, 77]. Nonetheless, this hypothesis needs to be examined as the signalling mechanisms of both Siglec-8 and CD300a in eosinophils have not been fully described and the actual function and necessity of the ITSM domain of Siglec-8 are currently unknown.

Given that eosinophils are recruited to inflammatory foci, the role of inhibitory receptors in the regulation of eosinophil migration has received some attention. Recruitment of eosinophils involves a signalling cascade where secreted chemokines interact with heterotrimeric G protein-coupled receptors (GPCRs) and especially with CCR3, the receptor for eotaxins. In response to eotaxin stimulation, CCR3 induces a signalling cascade that is accompanied by $Ca2^+$ mobilization and activation of Ras : ERK-dependent pathways and can associate with Src-family kinases such as Feline sarcoma viral (Fgr) kinase and haemopoietic cell kinase (Hck) [78]. Recent findings demonstrate a crosstalk between GPCR signalling and inhibitory receptors signalling. Specifically, the kinases Hck and Fgr possess a complex relationship with PIR-B. Although the precise pathway is not fully understood, it is known that Hck and Fgr phosphorylate the ITIMs of PIR-B, which in turn recruits SHP-1 and -2,

leading to subsequent dephosphorylation of yet unknown targets, resulting in the suppression of cell activation [38]. Hck and Fgr, as well as SHP-1 play key roles in the regulation of myeloid leucocyte migration. For example, neutrophils and dendritic cells that lack PIR-B or SHP-1 display enhanced chemokine signalling and functional responses, as do Fgr- and Hck-deficient cells [36, 38]. Given the proposed role for Hck and Fgr in CCR3-mediated eosinophil migration and their role in PIR-B-mediated activation, we were interested in defining the role of PIR-B in eosinophil chemotaxis. Surprisingly, we have shown that PIR-B may actually have a dual role in the regulation of eosinophil and neutrophil migration [33]. Importantly, Hck interacts with PIR-B in eosinophils as well but likely plays a different role than in neutrophils and dendritic cells as its association with PIR-B is relatively delayed kinetically and probably occurs following ITIM phosphorylation.

Interestingly, a novel role for Mig in the inhibition of murine eosinophil recruitment was recently demonstrated [79]. In their study, Fulkerson et al. [80] reported that the binding of Mig to CCR3, a hallmark eosinophil chemokine receptor, activates an inhibitory cascade (yet to be defined). Although this study was not conducted on a classical ITIM-bearing receptor, it suggests that different chemokines and perhaps other agonists can utilize CCR3 to inhibit eosinophil functions. Mechanistically, these findings could imply that a substantial crosstalk occurs between inhibitory receptors and 'eosinophil-specific' cytokine receptors such as CCR3.

Currently, there is only limited data on the role of inhibitory receptors in the down-regulation of allergic inflammation, which emphasizes the importance of further investigating their involvement in the various processes, such as integrin-signalling and migration.

Targeting inhibitory receptors as a therapeutic approach

Despite limited functional data on the function of inhibitory receptors on mast cells and eosinophils (compared with other cells such as NK cells and B cells), various approaches have already been taken to target such receptors as a therapeutic approach. Evidently, the fact that several independent groups have used similar strategies to target these receptors supports the notion that inhibitory receptors are potent targets for future drug design.

Specific targeting of mast cells (but not the general suppression of other myeloid cells) may yield a potent anti-allergic therapeutic approach. In the process of modelling, a therapeutic tool using inhibitory receptors, Saxon et al. have undertaken two approaches targeting inhibitory pathways in mast cells and basophils, which can be used either to treat chronic allergies independent of their allergenicity, or to specifically treat the patient with an antigen-dependent treatment [81, 82] (Fig. 4). One

platform, named GE2, uses negative signalling to generate a non-allergen-specific suppression of allergic reactivity. The additional platform, named GFD, uses a similar negative pathway but is actually Fel d 1 (a well-known cat allergen) specific. Both are based on a fusion protein that inhibits FcεRI-mediated responses by cross-linking it to FcγRIIB (GE2) or cross-links FcγRIIB with Fel d 1. Studies utilizing these reagents have shown promising pre-clinical results [81, 82].

As described previously, IRp60/CD300a is a potent negative regulator of mast cell and eosinophil responses [49, 75]. Bispecific antibody fragments, capable of recognizing IgE or CCR3 and CD300a/IRp60 (Fig. 4), were designed and administered *in vivo* in murine models of allergic peritonitis, passive cutaneous anaphylaxis and a chronic model of established allergic eosinophilic airway inflammation. The studies demonstrated a suppression of the allergic response and even a reversal of the inflammatory process and associated remodelling [83, 84]. Given the well-established role of FcγRIIB in the negative regulation of IgE-dependent mast cell activation, it is not
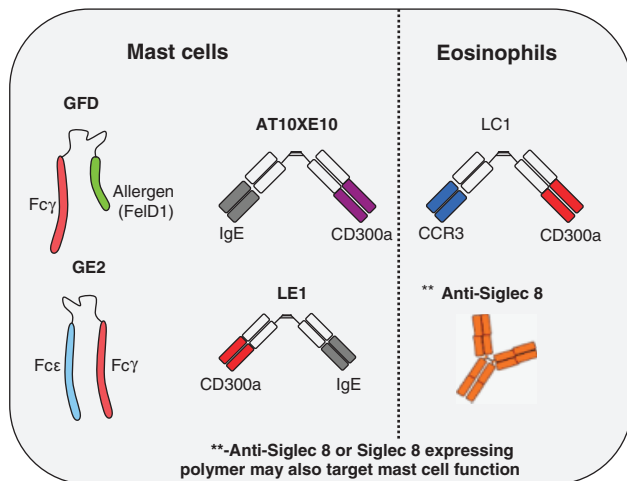

Fig. 4. Current strategies for targeting inhibitory receptors in allergic settings. The ability of inhibitory receptors to suppress mast cell and eosinophil activation has been exploited in an attempt to design future drugs. A bispecific fusion protein consisting of the Fc portion of IgG (Fcγ) and the Fc portion of IgE (Fcε) were linked (GE2). This fusion protein cross-links FcγRIIB to FcεRI and activates the inhibitory mechanism of FcγRIIB. Similarly, another fusion protein linking a major cat allergen (Fel d 1) to Fcγ was designed (GFD). This fusion protein mimics allergen binding to FcεRI portion and induces allergen-specific cellular inhibition. An additional approach that targets inhibitory receptors as anti-allergic drugs utilizes chemically conjugated bispecific antibodies that cross-link FcεRI-bound IgE to IRp60/Cd300a (LE1) or FcγRIIB (AT10XE10) and therefore activate the inhibitory mechanism of IRp60/CD300a or FcγRIIB. Similarly, a chemically conjugated bispecific antibody targeting the eosinophil-specific chemokine receptor (CCR3) and CD300a has been designed and shown to suppress eosinophil (and mast cell) activation *in vivo*. Finally, anti-Siglec-8 antibodies or multivalent artificial glycan ligands may provide a novel tool to suppress mast cell activation and eosinophilia in various allergic or hypereosinophilic syndromes. Siglec, sialic-acid-binding Ig-like lectin.

surprising that Tam et al have generated a bispecific antibody, which was used to cross-link FcεRI with FcγRII. Indeed, this bispecific antibody was capable to potently suppress mast cell and basophil activation [85]. Importantly, similar approaches have been taken to target inhibitory receptors on eosinophils in allergic-inflammation or states of hypereosinophilia (Fig. 4) [72, 73, 84, 86].

Thus, inhibitory receptors display vast potential in the regulation of cellular activation and therefore may serve as therapeutic targets in the future. Nevertheless and most importantly, when targeting these receptors, one should take into account various factors including cell specificity, general immune-suppression and the inflammatory context, which may induce ITIM-dependent co-activation rather than inhibition.

Concluding remarks and future perspectives

Despite ongoing progress in the field of inhibitory receptor function and signalling in mast cells and eosinophils, our understanding of these receptors' activities especially is still lacking. In this review, we have summarized some of the emerging data regarding inhibitory receptors in mast cells and eosinophils. Yet, further investigations studying these receptors are still needed. Such studies will likely

(a) Define the precise role of inhibitory receptors in various inflammatory settings involving mast cells and eosinophils.
(b) Define the molecular pathway, which mediate ITIM-dependent and -independent inhibition, especially defining the kinase complexes that can phosphorylate ITIM domains in these cells.
(c) Identify and characterize potential ligands for inhibitory receptors.
(d) Define the pathways that regulate inhibitory receptor expression in homeostasis and in inflammatory settings.

Addressing these questions will enhance our understanding on the molecular regulation of cellular function in health and disease. Furthermore, future studies may provide novel tools for future therapeutic approaches aimed to negatively regulate mast cell and eosinophil functions in allergic responses and/or other mast-cell- and eosinophil-related disorders.

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